An Aircraft Carrier Model for Long-term Gene Therapy

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ABSTRACT SUMMARY
A novel concept for sustain delivery of short interfering RNA (siRNA) into a target tissue, an aircraft model was designed. Solution of nano-polyplex with targeting ability turned into hydrogel after injection. And released nano-polyplexes circulate whole body and accumulate in the target tissue due to targeting moiety. Like an aircraft carrier, the nano-polyplex hydrogel released nano-polyplexes which induce gene silencing in long-term due to slow degradation of the hydrogel.

INTRODUCTION
Short interfering RNA (siRNA) is a double-stranded RNA molecule which can induce specific gene silencing by interaction with the mRNA. Delivery of siRNAs were studied to overcome some barriers such as the instability of siRNAs within serum and no ability to penetrate the cell membrane for application to treat diseases through gene silencing. In recent studies, the endowment of targeting ability to delivery carriers was reviewed to enhance the efficacy and reduce the side effects due to non-specific delivery. However, in most carriers even the targetable carriers for siRNAs, multi-injection was needed to maintain the effect in long-term. However, multi-injection is very inconvenient in patient’s perspective as well as time-consuming.

Here, we introduce the aircraft carrier model to give long-term targeted gene therapy due to siRNA delivery. As an aircraft carrier linger near the target region and warplanes go to attack the target, nano-polyplex hydrogel could be injected anywhere in the body and be sustained to release nano-polyplexes from the hydrogel circulating the whole body. Because the nano-polyplexes have targeting moiety, it could be accumulated in the target tissue selectively and induced gene silencing in only target tissue in long-term. To demonstrate our scheme, we conjugated folate, one of the targeting moieties to prepare polyethyleneimine (PEI) -conjugated poly(organophosphazene), (PP), cationic thermo-sensitive polymer which shows sol-gel transition in body temperature. After synthesis of folate-conjugated-poly(organophosphazene), (FPP), nano-polyplexes were induced by mixing with siRNAs and both in vitro, in vivo experiments were conducted.

EXPERIMENTAL METHODS
PP was synthesized by same method of the previous report. FPP was synthesized due to formation of amide linkage between carboxyl group of folate and primary amine of PEI. For in vivo targeting effect, the 1×10⁷ cells of MDA-MB-231 (folate receptor over-expressed) in 100µl of PBS were injected into dorsal subcutis of Balb/c nude mice (6 weeks, male, from Orient Bio, Korea). When the mean volume of tumors reached approximately 100 mm³ (Length×Width×Height×π/6), the solutions including nano-polyplex solution were subcutaneously injected. The intensity of cy3 was checked by Kodak Image Station 4000MM digital imaging system (Carestream Health, New Haven, CT, USA). For in vivo gene silencing test, after tumorigenesis using the same method, nano-polyplex solutions were administered by subcutaneous injection. Body weights and tumor volumes were monitored for 3 weeks at predetermined time intervals. The sizes of the tumors were measured using a caliper, and tumor volumes were calculated.

RESULTS AND DISCUSSION
We synthesized poly(organophosphazene), which was substituted with hydrophobic l-isoleucine ethyl ester (IleOEt), hydrophilic α-amino-α-metoxy-poly(ethylene glycol) (AMPEG), and 2-aminoethanol (AEtOH). Then we esterified the hydroxyl group of AEtOH to provide a hydrolysable ester linkage and a terminal carboxylic acid group. PEI was conjugated to the newly generated carboxylic acid group of poly(organophosphazenes) through an amide bond. Finally, folate was conjugated to amine group of PEI by amide linkage.

Figure 1. Cellular uptake of nano-polyplexes in FR positive and negative cell lines (A,B). In vitro VEGF gene silencing effect
After the characterization of FPP by 1H-NMR and GPC, temperature-dependent sol-gel transition of FPP solution was observed. Also we confirmed the complex formation of FPP and siRNA by zeta nanosizer.

To confirm the targeting effect of the nano-polyplexes, complexes between FPP and cy-3 tagged siRNA, we chose two different cell lines, MDA-MB-231 (Folate receptor positive) and A549 (Folate receptor negative). After treating the nano-polyplexes, only the group of MDA-MB-231 cells showed strong intensity within the cells. It means that FPP enter the FR positive cells selectively not the other cells. Gene silencing effect due to entering the siRNAs within cells was also checked by vascular endothelial growth factor (VEGF) quantification kit because we used siRNA for VEGF. Comparing with other group, lipofectamine, a commercial transfection reagent and FPP group showed gene silencing below 60–% and it could be explained that nano-polyplexes selectively enter the FR-positive cells and induce gene silencing via successful siRNA delivery. (Figure 1)

CONCLUSION

We developed an aircraft carrier model as a new concept for long-term targeted delivery of siRNA with only one injection. As siRNA could be used as a therapeutic material in various diseases, the new model could be expected to become an alternative method to cure most diseases including local diseases and systemic diseases.

REFERENCES


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